

CT features of Idiopathic Pulmonary Fibrosis correlated with Micro-CT and Histology

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Advances in Knowledge:

1. Micro-CT has shown to be a good tool to investigate parenchymal lung changes in IPF and can act as the until now missing bridge between HRCT and histology.
2. The process of lung fibrosis in IPF seems to start preferentially at the periphery of the secondary pulmonary lobule and grows inwards when disease progresses.

3. This study supports the theory that alveolar collapse might be the initial trigger for the fibroproliferative process in IPF.

Summary Statement:

There are elements in this study that suggest the presence of alveolar collapse in an early stage when there is only little fibrosis, supporting the theory that alveolar collapse might be the initial trigger for the fibroproliferative process in IPF.

Abstract

Purpose:

To better understand the underlying lung changes responsible for the CT features of idiopathic pulmonary fibrosis (IPF) and to gain insight into the way IPF proceeds through the lungs and progresses in time.

Material and Methods:

Micro-CT of tissue cores obtained from explant lungs were studied and correlated 1:1 with an immediate pre-transplant CT. Histology was obtained from selected cores.

Results:

Our study showed that in areas with no or minimal abnormalities on CT, small areas of increased density located in or close to the interlobular septa on micro-CT can be seen. In more involved lung areas, the number of densities increases, densities enlarge and approach each other along the interlobular septa which causes a fine reticular pattern on CT.

Simultaneously, air containing structures in and around these densities arise, corresponding with small cysts seen on CT. Honeycombing is caused by a progressive increase in number and size of these cystic structures and tissue densities that gradually extend towards the centrilobular region and finally replace the entire lobule. On histology, the small islands of increased density very likely correspond with 'fibroblastic foci'. Near these fibroblastic foci, an abnormal adjacency of alveolar walls was seen suggesting alveolar collapse. In later stages, normal lung tissue is replaced by a large amount of young collagen, as seen in advanced fibrosis.

Conclusion:

Fibrosis and cyst formation in IPF seems to start at the periphery of the pulmonary lobule and progressively extends towards the core of this anatomical lung unit. In this study, evidence was found that alveolar collapse might already be present in an early stage when there is only little pulmonary fibrosis.

Introduction

In 2002, the American Thoracic Society and European Respiratory Society set up an international multidisciplinary consensus classification of the different idiopathic interstitial pneumonias (1). This classification, which was updated in 2013 (2), is based on an integrated clinical, radiologic and pathologic approach and defines idiopathic pulmonary fibrosis (IPF) as a specific form of chronic fibrosing interstitial pneumonia with unknown etiology which is limited to the lung and associated with the histologic appearance of usual interstitial pneumonia (UIP). Imaging is stated to play a crucial role in the diagnosis of IPF with CT considered as a decision maker in multidisciplinary conferences (3,4). CT, indeed, provides important information by characterizing the entire lung at a (sub-) macroscopic level. CT has a high accuracy for a correct diagnosis when typical CT features are present, avoiding an invasive biopsy (5-7). This accuracy can be up to 95%, but drops significantly when typical signs are absent (8). Even then, CT may suggest the diagnosis in an appropriate clinical setting or may help to define when a biopsy would be more informative (1, 2).

Typical CT criteria for UIP are well established and include reticular opacities associated with traction bronchiectasis and honeycombing with a characteristic basal and peripheral distribution (1, 2). Although these signs were considered critical for the diagnosis, it is increasingly recognized that the CT presentation can be very heterogeneous and that a reticular pattern without honeycombing may be seen in UIP and may be sufficient for diagnosis in the proper clinical settings (9, 10). These CT changes in early UIP, and also those in more advanced UIP often present with a repeating almost geometric distribution pattern, suggesting pathological changes that expand by following the contours of a matching anatomic structure. Micro-CT was used to study these CT changes to determine the relationship between these abnormalities and the components of the secondary pulmonary lobule which is generally considered the basic CT anatomical unit of the lung parenchyma.

The purpose of this study is to better understand the underlying lung changes responsible for the CT features of idiopathic pulmonary fibrosis (IPF) and to gain insight into the way IPF proceeds through the lungs and progresses in time

Methods

Subjects:

Explant lungs from 10 consecutive white male patients with pre-transplant diagnosis of end stage idiopathic pulmonary fibrosis were selected. IPF diagnosis was made at a clinico-radiological-pathological conference and was based on the combination of clinical, radiological and, when a biopsy was available, pathological features. Postoperative pathological examination of the explant lungs was more suggestive for chronic hypersensitivity pneumonitis in one patient but confirmed the diagnosis of IPF in nine patients and these patients were used in this study. Their mean age was 57,22 years (range 51-64 years). Informed consent and permission to use these lung specimens were obtained directly from these patients. This study was approved by the hospital ethics and university biosafety committees in Leuven (S52174, MS20101571).

Specimen processing and sampling:

One explanted lung of each patient was donated for scientific research and numbered from one to nine. The explant lungs were prepared using the method described by McDonough *et al.* (11) and Verleden *et al.* (12). Briefly, after connecting the main-stem bronchus to a compressed air source with underwater seal, the lung is first inflated to a transpulmonary pressure of 30 cmH₂O and then deflated to a transpulmonary pressure of 10 cmH₂O. In this state, the lung is frozen in liquid nitrogen vapor, then stored at a temperature of -80°C.

The frozen lung specimens were cut into transverse slices of 2 cm thick from lung apex to base with a band saw. Cores of approximately 1.4 or 2.6 cm diameter were then randomly taken from each slice. These tissue samples were fixed in a 1% solution of glutaraldehyde in pure acetone (freezing point -93°C), precooled to -20°C and kept at that temperature during the fixation process. After the fixation, the samples were warmed to room temperature and washed through a graded series of ethanol concentrations (30%, 70%, 80%, 90% and 100% ethanol) followed by hexamethyldisilazane with samples air dried overnight (13).

Computed tomography (CT) scans:

The most recent CT before transplantation was used for correlation with micro-CT. The mean time between this CT and transplantation was 145,11 days (SD 149,82). On this CT also severity and extent of bronchiectasis, ground-glass opacity, consolidation, reticular pattern, cysts/honeycombing, pleural thickening, volume loss and air-trapping was estimated semi-quantitatively by 2 experienced chest radiologists (30 and 5 years of experience) using the method as described in De Jong *et al.* (14) These abnormalities were defined according to the Fleischner Society nomenclature (15). Briefly, each abnormality was scored in six lung lobes (lingula included), and per lobe the extent involved with the abnormality was estimated as less than one-third (score =1), between one-third and two-thirds (score = 2), and more than two-thirds of the lobar volume (score = 3). All scores were added and were expressed on a scale 0–100 because this scale may enable easier interpretation and comparison. The CT score can thus be interpreted as a percentage of the lung being involved.

CT of the explanted frozen lung specimens were obtained using a Siemens definition flash CT scanner at 120 kV, mAS modulation, collimation: 128x0.6 and pitch 1.2 with 1 and 5 mm slice thickness. These scans were used to facilitate the correlation between the preoperative CT and the micro-CT's.

The tissue cores were scanned with the SkyScan 1172 micro-CT scanner (Skyscan, Kontich Belgium) at a resolution of 8.4 μm , at 40kV and 250mA, without filter. SkyScanNrecon software (Skyscan, Kontich Belgium) was used to reconstruct the raw data.

A number of 2-20 cores per lung, with a total of 94 cores, were analyzed. These cores were selected from 1) areas that appeared normal on the pretransplant CT scan, 2) from areas with minimal abnormalities (small densities, linear opacities and early reticulation), 3) from areas with a clear reticular pattern and small cystic changes and finally 4) from lung areas showing progressive distortion and honeycombing. In this way between 20-24 cores within each of these 4 groups of CT were examined.

Correlation of preoperative CT, CT of the explanted frozen lung and micro-CT:

The micro-CT images of the tissue cores were registered by CM and JV with the preoperative *in vivo* CT images and with the explanted frozen lung CT using software developed within the ICON-IBBT AIR project (16) to exactly match the lung areas studied with micro-CT with the corresponding areas on the pretransplant CT scan. Care was taken not only to match the location but also the orientation and inclination of both scans.

Special attention was given to the location of the pathological changes in relation to the anatomy of the secondary pulmonary lobule.

Histology:

In each of the four above mentioned areas, cores were also selected for histology, which slices were taken and correlated with corresponding micro-CT and CT slices. Histology was performed on the cores after they were prepared as described in the specimen processing and sampling section. To find the corresponding slice within the core, the cores were first divided in 5 pieces (EV 30 years of experience). A classic hematoxylin and eosin stain (H&E stain) was performed on a representative slice of each piece. The piece that contains the interesting

slice was further divided and an H&E stain was performed every few cuts in order to find the slice of interest. Finally an H&E stain was also performed on the selected slices. It should be noted that these histological slices are different from those obtained through lung biopsy.

Because cellular structures were sometimes disturbed during processing, tissue characterization could be difficult. That is why additional picrosirius red staining was performed and viewed under a microscope with attached polarization filter. Polarized light microscopy was chosen because it has shown to be useful for assessing the maturity of collagen through the way fibers are organized. The picrosirius red staining enhances the polarizing features of collagen (17).

The theory is that in tissue scarring, as in fibrosis, young collagen fibers are deposited as fine disorganized fibers showing no or minimal birefringence (green), while native collagen is organized in thick bundles and shows strong birefringence, therefore appearing as bright yellow to red structures on a dark background in the polarizing microscope (17-19).

Results

All lungs showed typical CT features of UIP: an irregular linear to reticular pattern most pronounced in the basal and peripheral lung areas with peripheral cystic changes (honeycombing) and traction bronchiectasis in the basal parts of the lung (20-22). The scoring is presented in Table 1.

Invisible abnormalities on CT

Cores taken from regions that appeared normal on CT (Fig 1), and which were located in the apical and central areas, showed no changes on micro-CT. Figure 1b shows a micro-CT image of a core taken in an apical lung region, which appeared unaffected on CT (Fig 1a). A good

correlation is seen between CT and micro-CT images with corresponding anatomic structures (i.e. blood vessels and airways) recognizable on both scans. Figure 1 also shows that the interlobular septa are clearly depicted on micro-CT.

Ground-glass areas on CT

The amount of ground-glass opacification seen on the preoperative CT examinations in our study group is higher than would be expected in UIP patients, which is illustrated in the high ground-glass score (Table 1). This is probably related to the fact that our scoring method includes all areas of ground-glass opacity and doesn't discriminate between the ground-glass opacities that have been described in the very early stage of UIP, the increased density in the dependent lung due to hypoventilation or the increased density which is observed in the clearly abnormal and fibrotic regions. In our study ground-glass opacity on CT was mostly related to the latter condition and corresponded with areas of overt fibrosis on micro-CT. In figure 2a, however, a CT slice of lung 3 is presented where ground-glass opacity was seen in the dependent lung without overt fibrotic changes. The corresponding micro-CT was almost normal except for the presence of some small islands of increased density which, if followed through the core, were clearly adjacent to an interlobular septum. Histology was obtained at this level (Fig 2c-e) and the small island of increased density on micro-CT corresponded, predominantly, with abnormal and irregular strands of young polarizing collagen. In addition, polarization of native collagen was seen at the periphery of the lesion and in the surrounding normal alveolar walls.

Minimal changes on CT

In our study group, minimal changes on CT were often observed in the relatively spared upper regions of both lungs and consisted of small dots, small irregular linear densities, and

occasionally small cystic structures. Figure 3a shows a CT slice of the upper part of the left lung where a linear density and a cystic structure can be seen. On micro-CT (Fig 3b), the linear density corresponds with an added tissue density adjacent to a bronchovascular bundle and a nearby interlobular septum. The hypodense round structure is not a section through an airway, but an abnormal cystic structure next to a vein also located adjacent to an interlobular septum and is connected to alveolar ducts or sacs (Fig 3c-d). Adjacent to this cystic structure again some abnormal tissue densities can be seen. Figure 4a shows a micro-CT slice from another area of the same lung which was also minimally involved on CT. Again small, somewhat longer, and more irregular linear islands of increased density adjacent to the interlobular septum can be seen. H&E (Fig 4b) and picrosirius red (Fig 4c-f) stains were performed on this slice. The picrosirius red stain was viewed without (Fig 4c and e) and with polarization filter (Fig 6d and f). In one part of this linear density, bundles of strongly polarizing collagen orderly organized next to each other in a parallel direction can be seen (Fig 4d). This type of collagen is also seen in the alveolar walls. However, in the more irregular parts of this linear density, collagen fibers are fine, irregular and disorganized (Fig 4f).

Reticular pattern and small cystic changes on CT

Micro-CT of a core obtained in a peripheral lung region, where CT demonstrates linear densities that by summation start to resemble a net (a reticular pattern), reveals several islands of increased density located close to the interlobular septa (Fig 5). The secondary pulmonary lobule is outlined by these linear densities that together with the normal interlobular septa construct an almost hexagonal figure (Fig 5b) corresponding with the early reticulation on CT. At the center of the lobule, the terminal bronchiole and its accompanying arteriole can also be recognized and look normal. However, the alveolar structures surrounding some of

these islands of increased density have a distorted appearance. In Figure 5c-e, a terminal bronchiole is seen dividing into two first-generation respiratory bronchioles, which at their turn divide into second-generation respiratory bronchioles. Instead of giving rise to another generation of respiratory bronchioles or alveolar ducts, these second-generation respiratory bronchioles are terminating abruptly against islands of increased density.

When an obvious reticular pattern is seen on CT (Fig 6a), micro-CT shows the densities have become larger, broader and are interconnected resulting into broader bands following the course of the interlobular septa (Fig 6b).

A striking finding is that in some cores an important distortion of the alveoli and airways is found (Fig 7), while in other cores the architecture remains relatively undistorted even when the dense islands have grown into bands along the interlobular septa (Fig 6).

A common feature in these cores with islands of increased density is the presence of abnormal, irregular air containing structures in and near these dense islands (Fig 6, 8a-c). The presence of these air containing structures is independent of the size of the dense islands. They can already be observed early on, when only small islands of increased density are seen. They can be seen as small cysts on CT (Fig 3), but are often beyond the resolution of this technique.

Overt cyst formation or honeycombing

In the areas of the lung that are usually described as honeycombing with traction bronchiectasis on CT, the normal lung architecture and the secondary pulmonary lobule can hardly or not be recognized on micro-CT. Normal structures seem to be replaced by entirely dense areas containing traction bronchiectasis and multiple cystic structures of which only the largest are visible on CT (Fig 9 and 10). The cystic structures can sometimes be followed

throughout the core and seem to be connected with each other and are in continuity with the bronchial tree.

A picrosirius red stain of a slice with these dense areas (Fig 11) shows abnormal tissue with fine reticular green and yellow fibers under polarizing microscope, corresponding with young immature collagen.

UIP is known for its patchy geographical distribution with alternating normal and diseased regions in different stage of disease (1, 2). This is a histologic feature that can also be recognized on CT. CT in figures 9a and 12a shows a clear interface between what looks like a normal lung area and a predominantly cystic damaged lung region. This is confirmed on micro-CT (Fig 9b, 12b), although there is already some architectural distortion of alveolar structures adjacent to the large cysts.

Discussion

The geographical evolution of fibrosis in patients with IPF is largely unknown at this moment. Micro-CT was used to study progression of lung changes caused by IPF in order to better understand the CT features of this chronic lung disease. The sequence of CT lung changes is well known from previous *in vivo* CT studies. Akira *et al.* (23), studied serial CT findings in patients with IPF, seeing areas of ground-glass attenuation turning into a reticular pattern and ending with cystic changes. We examined different lung regions showing different CT features, which likely correspond with different stages of disease as is typically a heterogeneous disorder.

A first observation was that in areas with no or minimal abnormalities on CT a few islands of increased density can already be depicted on micro-CT that are beyond the resolution of CT. This confirms previous findings that patients with pathology proven IPF can present without the typical CT findings (24).

Pure ground-glass opacity, which can be seen retrospectively in very early stages of IPF was not frequently observed in our population of explanted lungs, very likely because our patients were transplanted due to end stage IPF. Most ground-glass that we observed, was located in the more affected regions and corresponded with very dense areas on micro-CT, probably overt fibrotic changes. One core taken in a ground-glass area at the periphery of the lung with no overt fibrotic changes, only showed some islands of increased density, which were too minor to be responsible for the global increased density on CT. A possible explanation is that the ground-glass seen on CT is the result of hypoventilation (dorsal area).

The reticular pattern on CT was related to the presence of more of these islands of increased density that were arranged at the periphery of the secondary pulmonary lobule and probably represents a later stage of the disease. In some cores cystic structures arising in and near these dense islands were seen corresponding with small cysts on CT. These abnormal cystic structures look quite similar to the tridimensional photographic reconstructions of 'honeycomb lungs' that Pimentel made in 1967 (25) and we think that these can be the precursors of the honeycomb cysts seen on CT. In addition in several cores micro-CT revealed small cystic structures that were also located in and around the densities at the periphery of the pulmonary lobule but that were not visible on CT, very likely corresponding with microscopic honeycombing (Fig 8a-c).

Finally, honeycombing seems to be caused by progressive enlargement of these cystic tissue densities that gradually extend towards the centrilobular region and finally replace the entire lobule.

Micro-CT was used as an intermediate imaging step between (sub)macroscopic CT images and histological microscopic images allowing a better correlation between the lung changes seen by both techniques. Histology at the different 'stages' shows that in the early stage the islands of increased density correspond with abnormal tissue with inlying young collagen,

probably fibroblastic foci. Near these islands/foci thick bundles of strongly polarizing collagen were seen. They look exactly like the native collagen in the alveolar wall, but with an unusual parallel organization. In the lungs we expect fine bundles of polarizing native collagen in the alveolar walls and interstitial space. The fact that they are in the immediate vicinity of each other and have the same orientation indicates increased approximation of alveolar walls, suggesting alveolar collapse. In the more advanced stages, these strongly polarizing collagen fibers are no longer seen, suggesting destruction of native alveolar structures and replacement by a fine reticular network of young collagen. This is typical seen in granulation tissue or early scar formation.

Much remains to be uncovered about the pathogenesis of IPF. An older hypothesis is that chronic inflammation plays a major role and that alveolar damage may possibly be the trigger for the fibroproliferative process (26). However, the current paradigm states that it is not the inflammation itself, but the epithelial damage that plays a crucial role in the pathogenesis of IPF (27-29). In 1988 Myers and Katzenstein (30) suggested that alveolar collapse following epithelial damage could be an important factor in lung remodeling. In fact, it is quite similar to epithelial damage to the airway wall, which could also lead to airway collapse or obliteration, as was recently proved by S. Verleden *et al.* (31) in bronchiolitis obliterans syndrome (BOS). Lately unifying theories have been provided by Leslie (32) and Chilos (33), suggesting that IPF is a disease of the aging lung, wherein chronic repetitive mechanical stress is exerted on regions prone to collapse in patients with a certain predilection. A recent work by Lutz (34) states that irreversible collapse is an important mechanism in lung injury and fibrosis.

In this study, there are elements on micro-CT and histology that support the ‘alveolar collapse theory’. We saw respiratory bronchioles terminating against the islands of increased densities and strands of polarizing mature collagen bundles approximating each other, both indicative

of tissue loss. This might be the trigger for the process of fibroproliferation, where normal native collagen is replaced by young immature collagen, seen as change of birefringence under polarized light. Second, when alveolar structures collapse, there might be traction on the surrounding lung structures, resulting in bronchiolectasis and the development of distorted cystic structures which are seen in the periphery of the secondary pulmonary lobule near the areas of increased density. As this process continues, there may be both increasing fibrosis and destruction of airways and airspaces with collapse, resulting in the typical image of IPF with macroscopic bronchiectasis and honeycombing.

Our results add to the understanding of the evolution of IPF. We succeeded showing what we believe are different stages of IPF on micro-CT and we were able to correlate them with the findings on CT. The way the different disease components relate to the secondary pulmonary lobule was also nicely depicted.

Our study has limitations. First, it is unavoidable that some changes might be introduced by the way the lung specimens are processed and by the way the tissue cores are prepared. Especially the freezing process might cause artifacts to the tissue. That is why histologic examination of these cores looks different from pathological UIP specimens obtained through biopsy. Second, we obtained histology from only a small section of the cores, so these results need to be confirmed with larger studies.

Conclusion

In conclusion, our study provides direct evidence that disease in IPF starts preferentially at the periphery of the secondary pulmonary lobule and grows inward as was suggested in an indirect manner before (35). We saw abnormal, cystic airway structures in the periphery of the secondary pulmonary lobule and believe that these are the precursors of honeycombing.

There are elements in this study that suggest the presence of alveolar collapse in an early stage when there is only little fibrosis, supporting the theory that alveolar collapse might be the initial trigger for the fibroproliferative process in IPF.

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Tables

Table 1: scoring of CT-signs on pre-transplant CT

Lung number:	1	2	3	4	5	6	7	8	9	Mean (\pm SD)
Bronchiectasis	44.4	61.1	100	66.6	19.4	69.5	36.1	88.9	16.7	56 (\pm 27)
Ground-glass	47.2	27.8	94.5	41.7	44.4	69.4	61.1	0	16.7	45 (\pm 27)
Consolidation	5.6	0	5.6	11.1	0	0	0	0	5.6	3 (\pm 4)
Reticular pattern	55.6	83.3	72.2	50	44.4	69.5	72.3	75	61.1	65 (\pm 12)
Cysts/ honeycombing	36.1	66.7	77.8	38.9	33.3	27.8	58.4	72.2	22.2	48 (\pm 20)
Pleural thickening	66.7	0	11.1	16.7	13.9	61.1	47.2	27.8	11.1	28 (\pm 23)
Volume loss	50	38.9	69.4	47.2	11.1	61.1	55.6	27.8	50	46 (\pm 17)
Air-trapping	11.1	/	/	5.6	/	5.6	13.9	11.1	/	9 (\pm 3)

All results are in %; / = no expiration scan was acquired.

Figure captions

Figure 1: No visible abnormalities on CT. (a) CT image of lung 1, at the apical part. The white circle indicates the region where the core is taken. The scale bar represents 1 cm. (b) Micro-CT of the core: normal blood vessels (black arrows) and interlobular septa (white arrows) can be recognized. There are no abnormalities on CT nor on micro-CT. The scale bar represents 1 mm.

Figure 2: Ground-glass areas on CT. (a) CT image of lung 3. There are ground-glass opacities in the dependent area of the right upper lobe. The scale bar represents 1 cm. (b) Micro-CT at this level shows some small areas of increased densities (black arrows). The scale bar represents 1 mm. (c) Corresponding histology slice of 2b (H&E stain, magnification x12). (d) Picrosirius red stain with magnification x100 of the indicated region in Figure 2c. (e) The same region as in d now using a polarizing filter. The density on micro-CT corresponds with abnormal tissue with a reticular network of fine green fibers (white arrows) with a few thicker strands of 'red' collagen at the periphery (black arrows).

Figure 3: Minimal changes on CT. (a) CT image of lung 1 (apical part). In the white circle a linear density (black arrow) with horizontal course is seen, possibly corresponding with an interlobular septum. A small cyst in the medial subpleural region can be seen (white arrow). The scale bar represents 1 cm. (b) On micro-CT, the linear density corresponds with an abnormal island of increased density (black arrow) adjacent to a bronchovascular structure and to a nearby interlobular septum (white arrowheads). The hypodense round structure (white arrow) is an abnormal cystic structure next to a vascular structure, probably a vein in an interlobular septum. Adjacent is some increased tissue density (black arrowhead). The scale bar represents 1 mm. (c) and (d) Close-up of the cystic structure seen in 3b at two different levels of the micro-CT core. On both levels connections with alveolar ducts and sacs are seen (white arrows).

Figure 4: Minimal changes on CT. (a) Micro-CT image of a core from lung 1 with islands of increased tissue density along an interlobular septum. The scale bar represents 1 mm. (b) H&E stain of this slice, magnification x12. (c-f) Picrosirius red stain without (c,e) and with polarizing filter (d,f), magnification x100. Native collagen in the alveolar walls shows strong birefringence and has a red color (white arrows). One part of the linear increased density (sample A: c,d) corresponds with thick bundles of the same red to yellow collagen orderly organized in close contact to each other (black arrows). Another sample (B, e,f) from a more irregular part of the linear density shows an added irregular reticular network of fine green fibers under polarization filter (white arrows).

Figure 5: Reticular pattern on CT. (a) CT image of lung 8 (upper part). In the white circle, linear densities organized as an interrupted reticular pattern can be seen. The scale bar represents 1 cm. (b) On micro-CT, the contours of the secondary pulmonary lobule (white arrowheads) with a terminal bronchiole and accompanying vessel in the center (white arrow) are shown. The linear densities on CT correlate with small islands of increased densities (black arrows) adjacent to these interlobular septa (white arrowheads) on micro-CT. The scale bar represents 1 mm.

(c-e) Close-up of the square in Figure 5b, where a terminal bronchiole (white arrowheads) is seen that divides into two first-generation respiratory bronchioles (white arrows). The inferior one divides into two second-generation respiratory bronchioles (black arrows) that abruptly terminate against an irregular island of increased density.

Figure 6: Reticular pattern and cystic changes on CT. (a) CT image of lung1 shows reticular pattern at the periphery of the lung (middle part). At this level the core is taken at the congruence of tree linear densities. The scale bare represents 1 cm. (b) On micro-CT we see that they represent dense bands along what is expected to be interlobular septa. There is minimal to no traction on the surrounding structures but several irregular cystic structures can be seen of which some are adjacent and some are in the dense bands (white arrows).The scale bar represents 1 mm.

Figure 7: Reticular pattern and cystic changes on CT. Micro-CT image of a core from lung 4. Islands and bands of increased densities (white arrows)are seen along the interlobular septa. The nearby alveolar structures show distortion (black arrow), possibly due to radial forces exerted on them by the densities. The scale bar represents 1 mm.

Figure 8: Cystic changes on CT. Micro-CT image of a core from respectively lung 1 (a), 4 (b) and 7 (c). Deformed cystic structures (white circles) are seen against and in islands/bands of increased density that are located along the interlobular septa. The scale bar represents 1 mm.

Figure 9: Overt cyst formation and honeycombing on CT. (a) CT image of lung 8 (middle part). The core is taken at a transition area between less involved and heavily involved and distorted lung. A distorted region with inlying cysts along a blood vessel is shown at the center of the circle. The scale bar represents 1 cm. (b) Micro-CT shows a large area of increased density containing multiple cysts. The cysts are better depicted on micro-CT and only the larger ones are visible on CT. The scale bar represents 1 mm.

Figure 10: Overt cyst formation and honeycombing on CT. (a) CT image at the base of lung 8. Cysts of different size in the periphery are seen. (b) Micro-CT shows the same changes as in figure 9, but with more and larger cyst. Adjacent to these cysts are areas of distorted alveoli and bronchioles (white arrows).The scale bar represents 1 mm.

Figure 11: Overt cyst formation and honeycombing on CT. (a) Micro-CT image of a core from lung 1.The scale bar represents 1 mm. (b) H&E stain of this slice, magnification x12. (c, d) Picrosirius red stain, magnification x100, without (c) and with (d) polarized light. A fine reticular network of green and yellow fibers is seen, compatible with young collagen fibers.

Figure 12: Interface between normal and cystic lung areas on CT. (a) On this CT image of lung 8 (middle-upper part), a clear cut interface between a normal area and a predominantly cystic region is seen which is typical for IPF. (b) On micro-CT there seem to be already some architectural distortion or compression of alveolar structures adjacent to the large cysts (white arrows).The scale bar represents 1 mm.